

**ION BINDING IN MICELLAR
SOLUTIONS, LIQUID CRYSTALS
AND BIOLOGICAL MODEL
MEMBRANE SYSTEMS STUDIED BY NMR TECHNIQUES**

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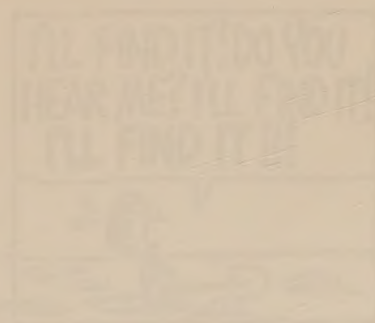


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NMR methods have become increasingly applicable to the study of interactions of small ions with various colloidal species in aqueous solutions. In most investigations of this kind, magnetic resonances of quadrupolar nuclei have been used in probing ion binding in for example biological tissues (1-3), protein solutions (4-6) and various amphiphilic systems (7-10). The desired information is usually obtained via NMR line widths or quadrupole relaxation times. Recently, it has also been shown that the chemical shift of alkali ions may provide a method to the study of ion binding in micellar solutions (11) and liquid crystalline phases (12).

In 1969 I started to study ion binding in micellar solutions using the quadrupole relaxation technique. These studies were later extended to include also lyotropic amphiphilic liquid crystals. The result of these investigations are reported in the papers (I-X) listed below, of which the following is a brief summary.

- I. G. Lindblom and B. Lindman. The Interaction between Halide Ions and Amphiphilic Organic Cations in Aqueous Solutions Studied by Nuclear Quadrupole Relaxation. J. Phys. Chem. 77, 2531 (1973).
- II. G. Lindblom, B. Lindman and L. Mandell. Effect of Micellar Shape and Solubilization on Counterion Binding Studied by ^{81}Br NMR. J. Colloid Interface Sci. 42, 400 (1973).

- III. G. Lindblom, B. Lindman and L. Mandell. A Study of Counterion Binding to Reversed Micelles by Nuclear Magnetic Quadrupole Relaxation of ^{81}Br . *J. Colloid Interface Sci.* 34, 262 (1970).
- IV. G. Lindblom and B. Lindman. Ion Binding in Liquid Crystals Studied by NMR. I. The Cetyltrimethylammonium Bromide-Hexanol-Water System. *Mol. Cryst. Liquid Cryst.* 14, 49 (1971).
- V. G. Lindblom, H. Wennerström and B. Lindman. Ion Binding in Liquid Crystals Studied by NMR. II. ^{35}Cl Second-Order Quadrupole Interactions in a Lamellar Mesophase. *Chem. Phys. Letters* 8, 489 (1971).
- VI. G. Lindblom. Ion Binding in Liquid Crystals Studied by NMR. III. ^{23}Na Quadrupolar Effects in a Model Membrane System. *Acta Chem. Scand.* 25, 2767 (1971).
- VII. G. Lindblom. Ion Binding in Liquid Crystals Studied by NMR. IV. ^{23}Na NMR of Macroscopically Aligned Lamellar Mesophases. *Acta Chem. Scand.* 26, 1745 (1972).
- VIII. G. Lindblom and B. Lindman. Ion Binding in Liquid Crystals Studied by NMR. V. Static Quadrupolar Effects for Alkali Nuclei. *Mol. Cryst. Liquid Cryst.* 22, 45 (1973).
- IX. H. Wennerström, G. Lindblom and B. Lindman. Theoretical Aspects on the NMR of Quadrupolar Ionic Nuclei in Micellar Solutions and Amphiphilic Liquid Crystals. *Chemica Scripta*. In press.

- X. N.-O. Persson, G. Lindblom, B. Lindman and G. Arvidsson. Deuteron and Sodium-23 NMR Studies of Lecithin Mesophases. Chem. Phys. Lipids. In press.

In the papers I-IV the ion binding has been investigated with the aid of quadrupole relaxation measurements, for micellar solutions (I-III) and for liquid crystals (IV). Quadrupole splittings have been studied in papers V-VIII and a theoretical basis for the interpretation of quadrupole relaxation times and quadrupole splittings is given in paper IX. In the last paper, X, an application of these methods to the study of ion binding in a lamellar liquid crystal of biological interest is introduced. Here also the water orientation at the amphiphilic surfaces has been investigated.

Amphiphilic compounds

An Amphiphilic molecule is characterized as possessing two moieties which differ greatly in their solubility properties. One of these moieties is hydrophilic and thus tends to be water soluble and insoluble in hydrocarbons and the other one is a hydrophobic group which tends to be water insoluble but soluble in hydrocarbons. Typical hydrophilic groups are $-OH$, $-O(CH_2CH_2O)_nH$, $-COO^-$, $-SO_3^-$ and $-N^+(CH_3)_3$ and typical lipophilic groups are saturated and unsaturated long-chain hydrocarbons. The dual nature of amphiphilic molecules is responsible for the formation of aggregates of different types, when the substance is dissolved in water. The aggregates are known as micelles and lyotropic liquid crystals. Due to the tendency of this type of amphiphiles to become adsorbed at interfaces, they are often called surface-active agents or colloidal surfactants. The majority of the work summarized here has been done on such

amphiphiles. Examples of amphiphiles studied are alkylammonium compounds, alkali soaps and lecithins.

Micelles and Solubilization

At low concentrations the amphiphilic molecule behaves like simple electrolytes i.e. it is completely dissociated, and its physical properties approach ideality at infinite dilution.

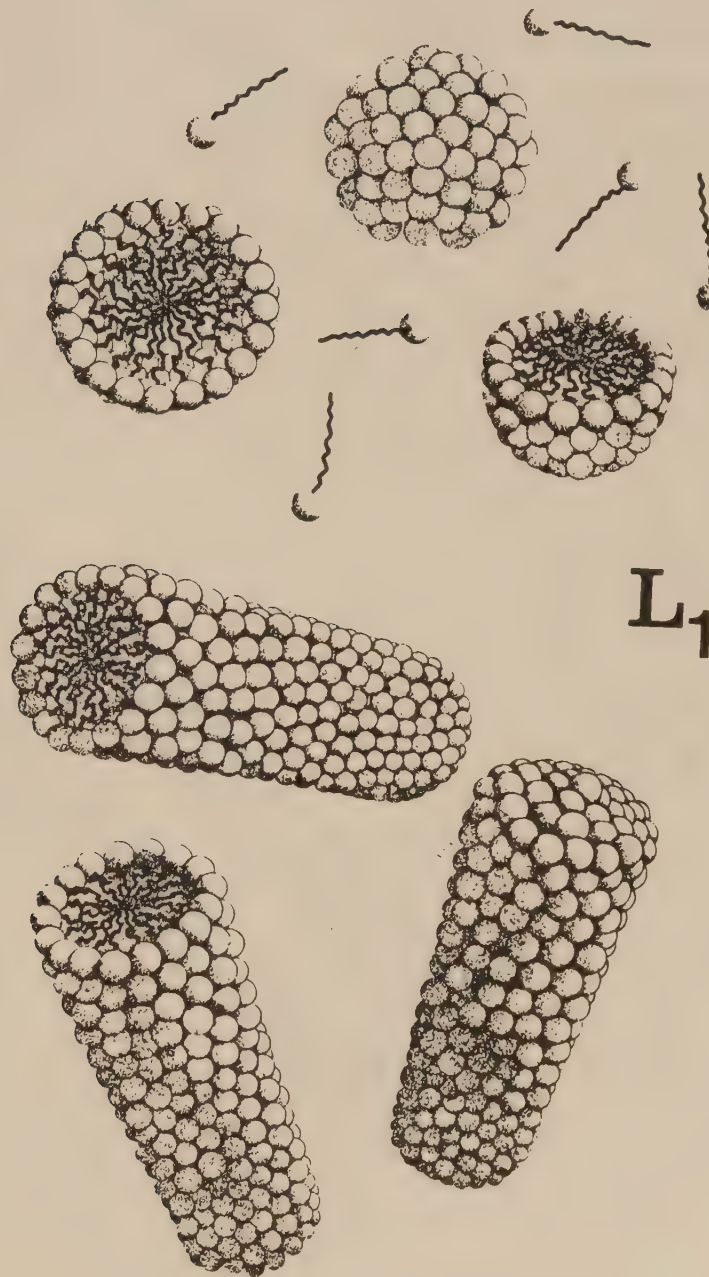


Fig. 1. Schematic representation of rod-like and spherical micelles.

At higher concentrations and above a certain temperature, termed the Krafft point, the amphiphilic molecules associate in large aggregates called micelles. For an ionic amphiphile dissolved in water, these micelles can be considered as consisting of a hydrocarbon core with the polar head groups facing outwards towards the surrounding aqueous solution. This is an example of the normal type of micelles, and a schematic representation of micelles is shown in Fig. 1. The shape of a micelle may vary for different amphiphiles (Spheres, ellipsoids, or long cylinders) and often depends on concentration and temperature. Additionally, another type of micelles may form, when the solvent is a liquid organic compound, namely the so-called reversed micelle. Here the water forms the core of the micelle and the alkyl chains face outwards towards the solvent (Fig. 2).

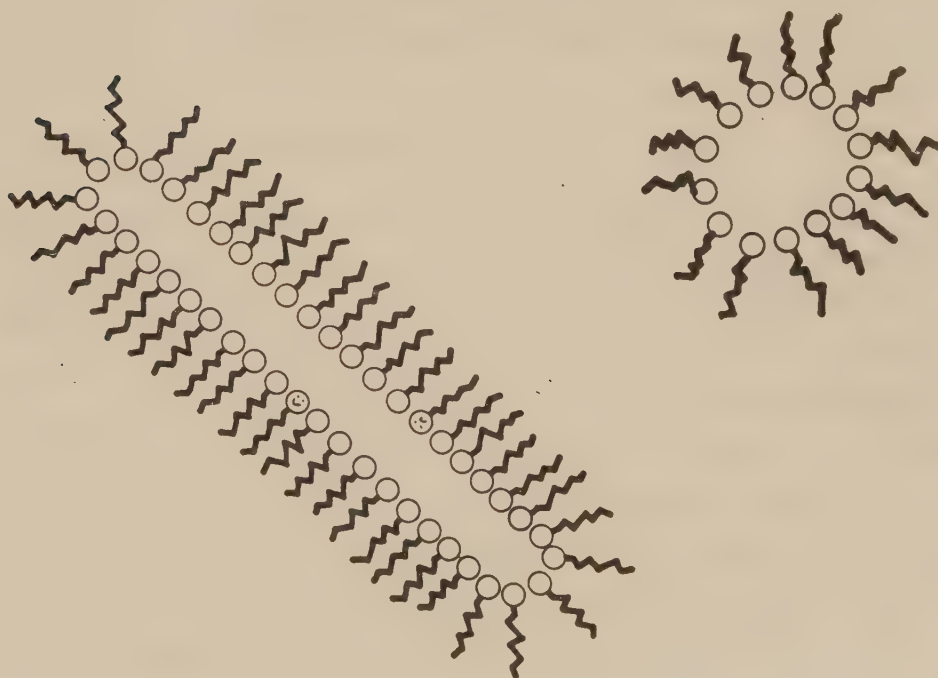


Fig. 2. Schematic representation of reversed micelles.

Amphiphilic solutions possess certain striking solubility characteristics. For example an aqueous amphiphile solution above the critical micelle concentration (cmc) can dissolve various organic compounds which in the absence of amphiphile are insoluble or very slightly soluble in water. This phenomenon was termed "solubilization" by McBain and McBain (13). Similarly, amphiphiles like Aerosol OT or lecithins, often called "swelling amphiphiles", when dissolved in a hydrocarbon are able to solubilize water or glycerol which are insoluble in the hydrocarbon in the absence of the amphiphile. Solubilization has been studied with many physical methods. These include conductivity measurements, light scattering, X-ray diffraction and proton NMR. Recent reviews about solubilization and its applications have been written by Elworthy, Florence and Macfarlane (14) and by Ekwall, Mandell and Fontell (15). Paper II of this thesis gives an application of NMR quadrupole relaxation to the study of the location of solubilizate in the micelles.

Amphiphilic mesophases

Amphiphilic mesophases or lyotropic liquid crystalline phases can be obtained at a certain temperature primarily in two ways, by increasing the amphiphile concentration or by adding a solubilizate. When for example the concentration of an aqueous solution of cetyltrimethylammonium bromide (CTAB) is increased above about 27% (w/w) a mesophase called the middle phase or E-phase forms (16). Alphabetical notation used by Ekwall and co-workers (15) will be employed. X-ray data (16) obtained from this mesophase have been interpreted to give a structure shown in Fig. 3.

If hexanol is dissolved in a CTAB micellar solution another mesophase, the neat or D-phase, may form (16).

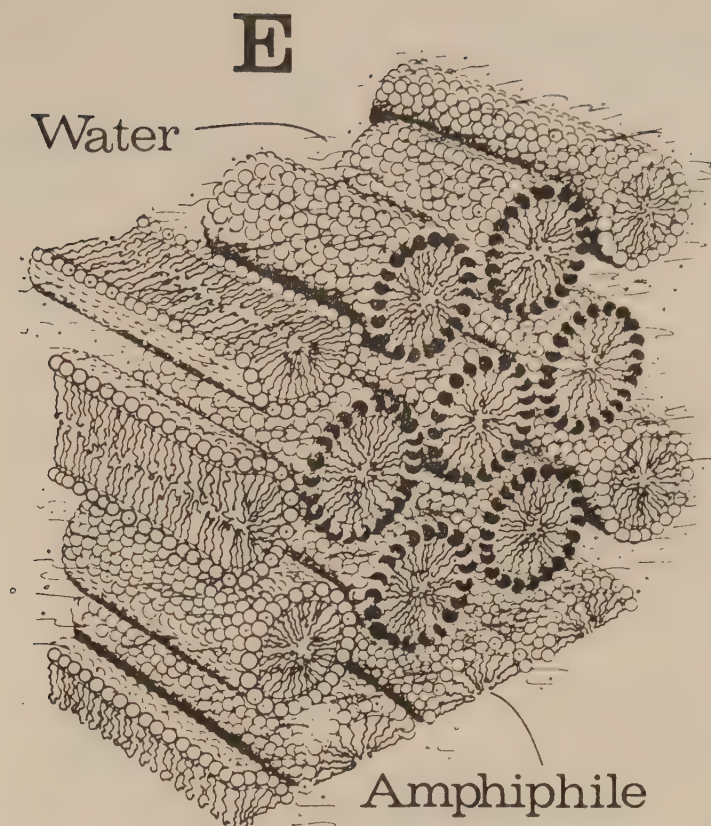


Fig. 3. Schematic representation of the packing pattern of rod-like particles in a two-dimensional hexagonal mesophase.

The typical Bragg spacing ratio $1:1/2:1/3$ obtained from the X-ray diffraction pattern of this phase can be interpreted in terms of a structure having coherent double layers of amphiphile molecules with the polar end-groups in the interface with intervening layers of water molecules (Fig. 4).

The work considered in this thesis has been performed with amorphous solution phases and liquid crystalline phases of a number of ternary amphiphilic systems. Ekwall and co-workers have presented

in a series of papers systematic investigations of the phase equilibria at constant temperature in binary and ternary systems of amphiphile, water and an organic compound (15, 17). The phase diagrams used in this work are given in the appendix.

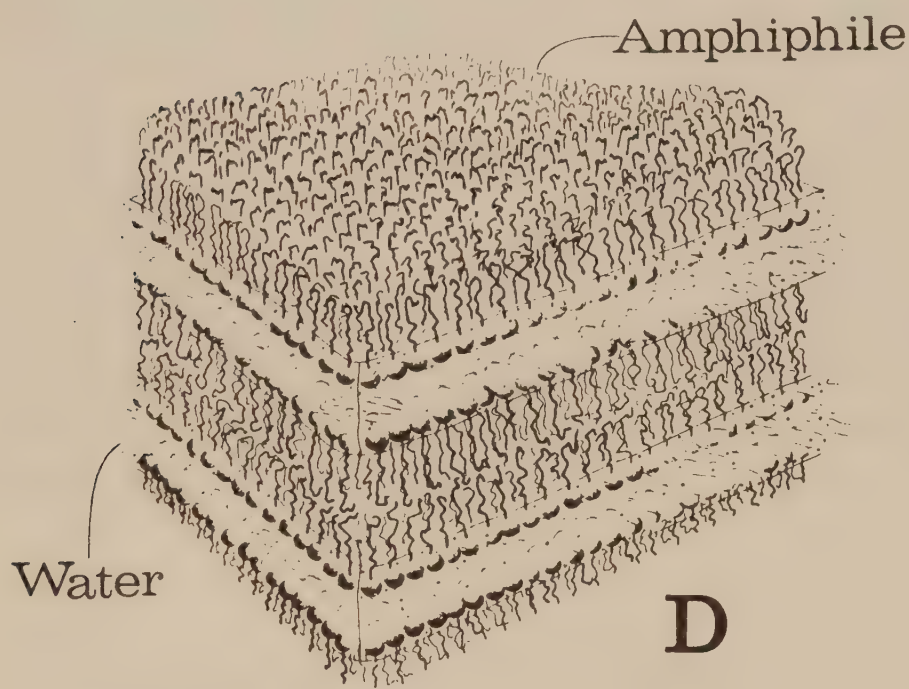


Fig. 4. Schematic representation of the structure of the lamellar mesophase D.

Biological membrane systems

One of the most interesting properties of the cell is that it seems not to be considerably disturbed by changes in its environment and that some substances are allowed to enter the interior of the cell but others are not. This property of the cell

is maintained by the cell membranes. These membranes are composed of proteins and lipids, with a composition varying from one type of cell to the other. For example the myelin membrane consists of 20% protein and 80% lipid while the composition of the erythrocyte membrane is 49% protein and 43.6% total lipid (18). Expectedly, the proteins are different for different membranes but also the lipid composition varies considerably (18).

It has become increasingly clear that the lipids in biological membranes form a bilayer matrix in which the proteins in some way are embedded (19). Several membrane models based on a lipid matrix have been constructed (19). As an example the model proposed by Singer (20), in which the protein molecules are simply inserted into the lipid bilayer matrix is shown in Fig. 5.

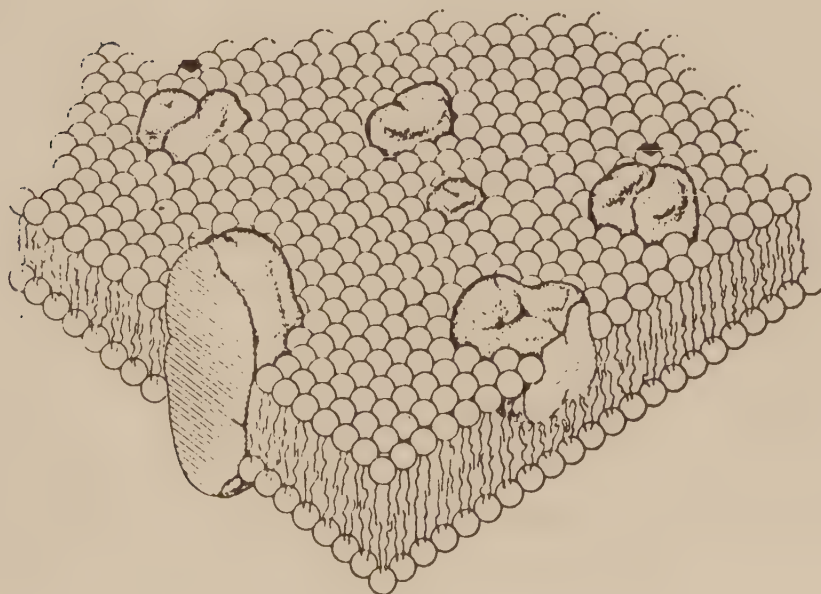


Fig. 5. A schematic representation of the three-dimensional organization of the mosaic structure with the phospholipids forming the matrix of the membrane. The globular protein molecules are partially embedded in the membrane, and partially pro-

It is supposed that the regions of the bilayer at some distance from the proteins behave as an essentially unperturbed lipid bilayer having properties similar to lamellar liquid crystals containing phospholipids.

The liquid crystalline state of the membrane lipids have stimulated many membrane investigators to study phospholipid mesophases like lamellar lyotropic liquid crystals, lecithin vesicle (liposom) solutions and also monolayers of lipids. The use of phospholipid bilayer or monolayer membrane models gives the opportunity to investigate how membrane proteins, enzymes, cholesterol, ions like Na^+ , K^+ , Ca^{2+} and Mg^{2+} , water etc. interact with lipids, and how the interaction affects the phase structure and the function of the constituents.

Quadrupolar relaxation

For nuclei which possess electric quadrupole moments, eQ , a generally very powerful relaxation mechanism is afforded by the interactions of the quadrupole moment with fluctuating electric field gradients, eq . For such nuclei, this interaction is usually the dominant relaxation mechanism. No well-defined relaxation times (T_1 or T_2) exist for nuclei with spin quantum numbers, $I > 1$ unless the molecular motion is rapid with respect to the resonance frequency at which the measurement is made. However, for counterion relaxation in micellar solutions this is usually the case and the inverse quadrupole spin relaxation times, T_1 and T_2 , are equal and given by the expression (21)

$$\frac{1}{T_1} = \frac{1}{T_2} = \frac{3}{40} \frac{2I + 3}{I^2(2I - 1)} \left(1 + \frac{\eta^2}{3}\right) \left(\frac{e^2 q Q}{h}\right)^2 \tau_c \quad (1)$$

Here η is a measure of the deviation from cylindrical symmetry of the electric field gradient tensor and τ_c is a correlation time which determines a time scale of the molecular motion. For normal liquids τ_c is of the order of 10^{-11} - 10^{-12} s. T_2 is related to the NMR line width, ΔB between maximum slopes of the absorption curve by

$$\Delta B = \frac{2}{\sqrt{3}\gamma T_2} \quad (2)$$

for a Lorentzian line shape. Thus the NMR line width will depend upon τ_c and upon the strength of the quadrupole coupling. For ions in solution, there is a problem to decide to what motion in the system studied the correlation time refers. The situation is further complicated by the absence of any knowledge of the quadrupole coupling constant. Consequently, a quantitative interpretation of line widths of counterions in amphiphilic systems is difficult. However, for studies of ion binding in micellar solutions a qualitative interpretation of line widths often suffices for many purposes.

It was first shown in a preliminary report by Eriksson et al. (7) that the ^{81}Br quadrupole relaxation rate increased markedly above the cmc for an octylamine hydrobromide solution, indicating a binding of counterions to the micelles. In order to obtain a better understanding of the nature of the ion binding to different charged amphiphilic surfaces, we applied the quadrupole relaxation method to studies of the interaction of counterions with various amphiphilic systems (I-IV).

A detailed description of the counterion binding to the aggregates can, however, only be given if the processes causing relaxation can be determined. Let us therefore start with a discussion of the factors that determine the relaxation rate in the systems considered. These are the correlation times,

the field gradients and the distribution of counterions among the different sites in the system (I).

For a qualitative treatment of the ion binding in micellar solutions and mesophases a model with only two sites namely, free counterions and counterions attached to the amphiphilic surface, is very useful (I-IV). In this case the fraction of ions bound may be estimated from observations with other methods. A rigorous quantitative treatment does not permit such a simple model, since the ions may be bound to the amphiphilic surface in different ways, e.g. both with and without hydration water.

It has not been possible to distinguish between different binding sites at the amphiphilic surfaces, but evidence that the main part of the bound ions are hydrated has been obtained (I, III): the addition of small amounts of water to a solution of cetyltrimethylammonium bromide dissolved in hexanol considerably reduces the ^{81}Br line width, indicating that hydration of the ion pairs is important. Further addition of water leads to the formation of reversed micelles, where the line width is almost constant over a wide range of concentrations. Here the line width approaches the counterion line width obtained in the normal micellar solution (II). In paper I a simple salt titration experiment shows that about 80% of the counterions are bound in some sense to the micelles. The same degree of association of counterions has been obtained by studies using other methods (22, 23).

An estimate of the electric field gradients and the correlation time is more difficult to make. From an examination of the ion binding for various alkyltrimethylammonium halide solutions below and above the cmc it is concluded (I) that changes of the field gradients are important factors in the relaxation mechanism. The following observations led to this conclusion.

a) Below the cmc, ^{81}Br and ^{35}Cl NMR line widths are greater for amphiphiles having a $-\overset{+}{\text{N}}(\text{CH}_3)_3$ polar head group than for amphiphiles with a $-\overset{+}{\text{N}}\text{H}_3$ head group.

b) Above the cmc, a reverse dependence of the line widths on the head groups occurs.

In a series of papers by Lindman and co-workers (24-26) it has been observed that for solutions of small organic cations, where formation of micelles is unlikely to occur, the line widths increase markedly with increasing concentration and with increasing size of a symmetrical cation. This is explained in terms of an incorporation of part of the anions in a modified water structure in the vicinity of the alkyl chains. Since the structure-making effect is larger for N-methylsubstituted alkylammonium cations than for alkylammonium cations (27) this explains the difference of the line widths below the cmc for the two end groups considered.

Above the cmc the simple two site model was used to calculate a line width related to the micellar ion binding site. (This can be considered as an average line width for different possible binding sites at the micellar surface.) Since, for a given length of the carbon chain, the counterion relaxation is more effective for alkylammonium micelles than for alkyltrimethylammonium micelles it is concluded that the micellar surface charge is significantly effecting the relaxation, i.e. an increase of electric field gradients at the micellar binding site causes the increased relaxation. Unfortunately, it is difficult to decide whether these field gradients arise from water molecules or the amphiphile polar groups (IX).

Finally, we have to consider which motional process(es) causes the relaxation. In paper IX estimates of the relaxation rate for different motional models are presented and the general conclusion is that an electrostatic model may explain the relaxation rate

observed for micellar solutions. It was also shown, that both a fast local motion and an overall slow motion give calculated relaxation rates comparable to the experimental ones. However, since the counterion line widths in most cases change to a very small extent at phase transitions (II, IV, 28), it is concluded that the fast local motion is most important in determining the relaxation rate. Only for one case (II) a significant decrease in the line width has been observed in going from a micellar solution to a mesophase and this observation is difficult to explain if the slow motion does not contribute to the relaxation rate in the micellar solution. It should, however, be noted, that since the decrease of the line width is rather small the slow motion is effecting the relaxation only to a minor extent.

In paper IX it is also shown that a local motion like a translation of the counterions perpendicularly to the micellar surface may be responsible for the line width observed for micellar solutions. (The assignment in paper I of the relaxation mechanism to water molecular motion is not unassailable.)

In spite of the fact that many problems for a quantitative interpretation of counterion relaxation data remain to be solved, the quadrupole relaxation method can be successfully used to obtain qualitative information on ion binding. The strength of the method lies mainly in its considerable sensitivity to changes of the charge densities at the amphiphilic surface. It is therefore expected that this method, besides being a convenient method for the determination of cmc values (I, III) can be used to detect e.g. transitions from globular to cylindrical micelles (II) and location of solubilized organic molecules (II, IV). In paper II it is shown that a transition from globular to rodshaped micelles is taking place at about 10% CTAB at 27°C and that globular micelles

are favored at higher temperatures. It is interesting to note that no such transition seems to occur for cetyltrimethylammonium chloride (CTAC) solutions. Observations from other methods support these conclusions (29). It should also be noted that no competition is observed between bromide and chloride ions in mixed CTAB and CTAC solutions (II). A possible interpretation of these results is that both globular and rod-shaped micelles exist in concentrated mixed amphiphile solutions. Since the difference in the viscosity is very large between solutions of globular and rod-shaped micelles a measurement of the viscosity for CTAB, CTAC and CTAC+CTAB solutions might give valuable information in this respect.

Solubilization experiments described in paper II show that hexanol, benzene and N,N-dimethylaniline are located at or near the micellar surface, causing a decrease of the charge density which results in a partial release of bromide ions from the micelles. On the contrary cyclohexane is solubilized in the interior of the micelles. These findings are in agreement with proton NMR studies (30).

The effect of solubilization of hexanol on the ion binding in a lamellar mesophase is presented in paper IV. The results are qualitatively the same as those obtained in micellar solutions (II). It should be noted here that a problem may arise when studying line widths in anisotropic mesophases, since static quadrupole effects of first or second order (IX, 28) may affect the line shape. This is not always easily detected by visual inspection of the NMR spectra (28). Thus, to be able to give an unambiguous interpretation of line width data obtained in anisotropic liquid crystalline phases it is necessary to carefully test for the presence of static quadrupole effects, e.g. by an investigation of the frequency dependence of the line width (V) and an examination of the intensity of the NMR signal.

In the CTAB-hexanol-water system, a first order ^{81}Br quadrupole splitting of 200 kHz has been observed in the hexagonal phase (II); however, in the lamellar mesophase no splitting could be observed, presumably due to a low signal-to-noise ratio. It is shown in paper IX that the splitting for a lamellar phase theoretically should be twice that of a hexagonal one provided the molecular interactions are the same in the two phases. From the splitting for the hexagonal phase it is then possible to estimate if static effects of second order influence the line width obtained in the lamellar phase. A straight-forward calculation using appropriate formulas given in paper IX shows that static quadrupolar interactions may have an effect on the observed line width. Since for a sample of the lamellar phase considered no frequency dependence of the line width could be found, it is reasonable to assume that the effect of static quadrupole interactions is small.

Quadrupole splittings

Static quadrupole effects are observed in solids (29) and also in anisotropic liquid crystals. The interaction between the quadrupole moment, eQ and electric field gradients, eq shifts the energy levels of the spin states. In the limit where the nuclear Zeeman term in the hamiltonian is much larger than the quadrupole interaction we can calculate (31) the energies to first order for $I=3/2$ to be

$$E_{3/2} = -3/2 \, h\nu_L + 3/4 \, Af$$

$$E_{1/2} = -1/2 \, h\nu_L - 3/4 \, Af$$

$$E_{-1/2} = 1/2 \, h\nu_L - 3/4 \, Af$$

$$E_{-3/2} = 3/2 \, h\nu_L + 3/4 \, Af$$

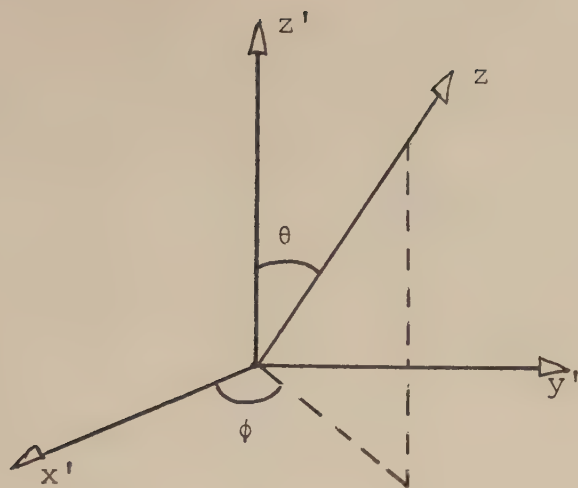


Fig. 6. Coordinate system showing the spherical coordinates θ and ϕ of the z -axis of the laboratory frame in the quadrupolar principal axis system $x'y'z'$. The laboratory coordinates z and x are defined by the magnetic field and the NMR transmitter coil axis, respectively.

Here it has been assumed that the external magnetic field, B_0 , is in the z direction (see Fig. 6). ν_L is the Larmor precession frequency,

$$A = \frac{e^2 q Q}{6} \quad \text{and} \quad f = 3\cos^2\theta - 1; \quad (\eta = 0).$$

These energy levels are illustrated for $Af > 0$ in Fig. 7, where also the corresponding NMR spectra are shown. It should be noted that the $-1/2 \rightarrow 1/2$ transition frequency is independent of the quadrupole interaction to first order. The relative magnitude of the three resonance lines is governed by the transition probabilities between the different energy levels. It turns out (31) that the central line contributes 40% of the total intensity while each of the satellites contributes 30%. We define an experimental quantity, which we call the quadrupole splitting, $\Delta_{\theta'}^{(1)}$, as the distance (in Hz) between satellites obtained in the NMR spectrum, corresponding to the distance z - B in Fig. 7. For a liquid crystalline sample $\Delta_{\theta'}^{(1)}$ can be written

$$\Delta_{\theta'}^{(1)} = |p\nu_Q S(3\cos^2\theta' - 1)| \quad (3)$$

where p is the fraction of nuclei in a site with an asymmetric environment giving rise to static quadrupole interactions.

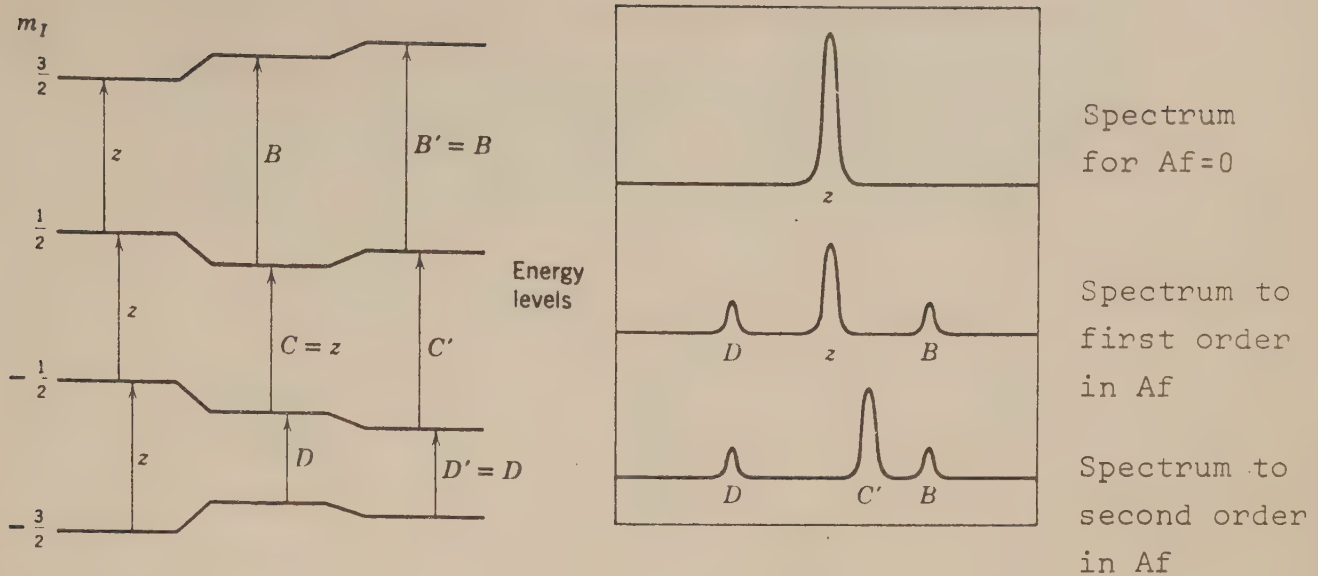


Fig. 7. Energy levels for $I = \frac{3}{2}$ with $m_I = \pm \frac{1}{2}, \pm \frac{3}{2}$, showing NMR transitions in the absence (left), with a first-order quadrupole shift (center), and with first- and second-order shifts included (right). The allowed transitions ($\Delta m_I = \pm 1$) are indicated, and the corresponding spectra are shown.

ν_Q , the quadrupole coupling constant, represents the strength of the interaction between the electric field gradient and the quadrupole moment. S , the order parameter, describes the degree of orientation of the electric field gradient along a certain axis of symmetry, often called the director, θ' is the angle between B_0 and the director. When a polycrystalline material or an unaligned mesophase is employed the spectral shape of the NMR signal shows a typical powder pattern (Fig. 8). This spectral shape is obtained since, for a powder sample, the randomly oriented crystals or microcrystallites give rise to a superposition of resonance signals over all angles. The quadrupole splitting, $\Delta_p^{(1)}$, for an unaligned liquid crystal is given by

$$\Delta_p^{(1)} = |p\nu_Q S| \quad (4)$$

A pronounced second-order correction to the energy shifts the central line, as is shown in Fig. 7.

A typical powder pattern of the central line caused by the second order shift in the NMR spectrum is illustrated in Fig. 8.

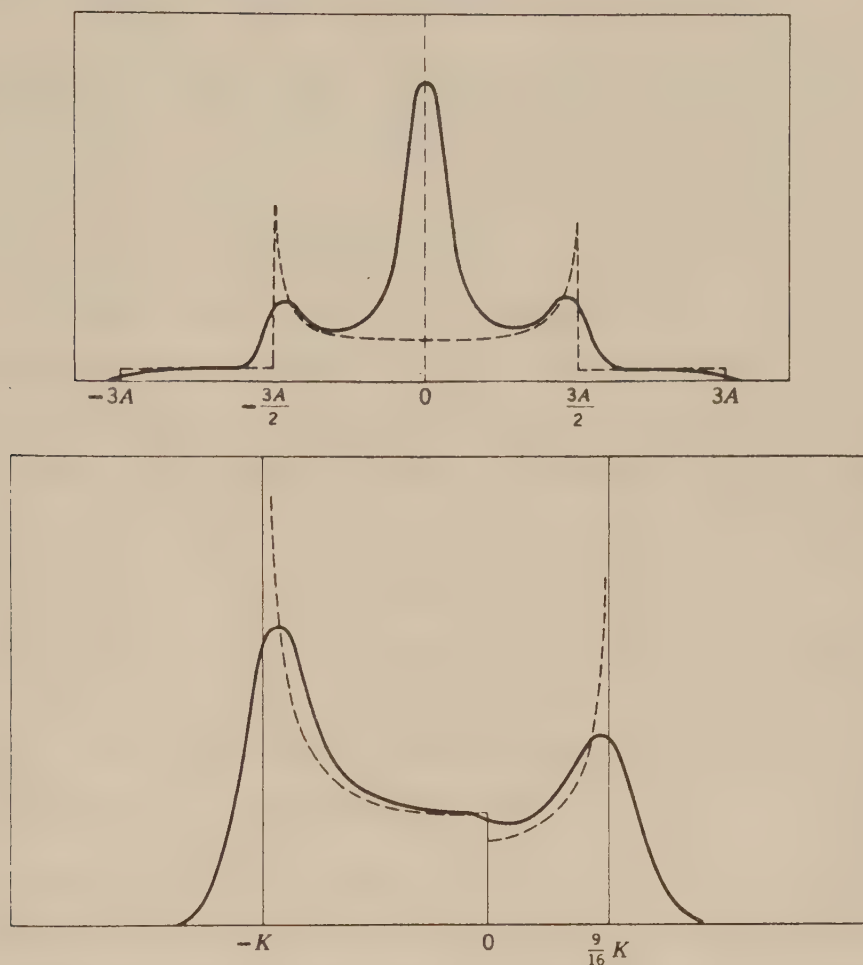


Fig. 8. Powder patterns for symmetric field gradients ($\eta=0$) with $I=3/2$, showing first-order pattern (top) of the overall spectrum and second-order pattern (bottom) of the central component. The spectra are shown relative to the center point where $\nu_L = \gamma B_0 / 2\pi$ with $A = e^2 q Q / 6$ and $K = 3A^2 / \gamma \hbar B_0$. The dashed curves are for infinitely narrow single-crystal lines, and the solid curves are for dipolar broadened single-crystal lines, for the case $|\gamma \hbar B_0| > |A|$.

As may be inferred from Fig. 8 the shift term gives rise to a "splitting" of the central peak. We define the distance between the peaks, as measured from the derivative of the absorption signal in the NMR spectrum, as the second order quadrupole splitting $\Delta_P^{(2)}$

$$\Delta_P^{(2)} = \frac{25}{48\nu_L} (p\nu_Q S)^2 \quad (5)$$

Quadrupole splittings of first and second order in NMR spectra of counterions in mesophases were first reported in papers V and VI. In the lamellar phase composed of octylammonium chloride, decanol and water an anomalous ^{35}Cl NMR line was observed. In paper V we conclude from an investigation of the frequency dependence of the NMR signal together with a simulation of the line shape and by a comparison of the ^{35}Cl and ^{37}Cl spectra that the anomalous line shape obtained is due to second order quadrupole effects. Since the second order quadrupole shift is inversely dependent on the Larmor frequency (cf. Eq. 5), which is not the case for first order quadrupole effects, cf. Eq. 4, a study of the frequency dependence of the shift gives unambiguous experimental verification of second order quadrupole effects.

My interest in biological membranes led me to attempt a study of ion binding in a simple model membrane, a lamellar mesophase containing water, egg yolk lecithin and sodium cholate. In this system a first order ^{23}Na quadrupole splitting is observed (VI). Later also other authors have reported first order ^{23}Na quadrupole splittings of counterions in different anisotropic mesophases (32, 33). Shporer and Civan observed sodium splittings in a lamellar phase of sodium linoleate in water. These authors also draw attention to the difficulties of using the observed NMR signal intensity for studies of the fractions of bound sodium ions in biological tissues. Remarkably often, only about 40% of the total ^{23}Na signal was observable in such systems (34). As has been pointed

out by Shporer and Civan these findings do not necessarily reflect division into "bound NMR-invisible and free NMR-visible" sodium ions, since static quadrupole interactions may be expected to occur.

In order to examine the potential use of quadrupole splittings as a method to study ion binding in anisotropic lyotropic liquid crystals and biological model membrane systems we have started a systematic investigation of alkali and halide quadrupole splittings. Similarly, as for the quadrupole relaxation method, it is difficult to extract quantitative information on ion binding from a study of quadrupole splittings. Since the splitting depends on the order parameter, Eqs. 4 and 5, there is a difference between the two methods concerning the qualitative information that can be obtained. It is expected that the quadrupole splitting method can be used to detect changes in the geometric disposition of the charged amphiphilic surface (IX, X, 35).

In paper VII an application of the method to the study of a macroscopically aligned lamellar mesophase is described. The observed quadrupole splitting for the aligned sample was proportional to the function $(3 \cos^2 \theta - 1)$, cf. Eq. 3, indicating that the lamellae of the mesophase are oriented along the glass plates employed to give macroscopic alignment. This technique may be useful for detection of curved lamellae in mesophases.

The quadrupole splitting method can be conveniently used for detection of phase transitions (VIII, 36). For example it is observed that the phase C composed of sodium octanoate, decanol and water gives a rather large splitting in the ^{23}Na NMR, whereas for unaligned samples at nearly the same composition, for the lamellar phase no splitting is observed. It has also been observed (35) that for a lamellar mesophase composed of dimyristoyl lecithin (DML) and 0.8 M NaCl aqueous solution, the ^{23}Na splitting is almost con-

stant below 23°C , i.e. in the gel phase (18), but that the splitting increases with increasing temperature in the liquid crystalline phase above 23°C . The deuteron quadrupole splitting from heavy water in lecithin-containing systems exhibits a sharp minimum at the Chapman transition temperature (35, 37).

The effect of cholesterol on the ^{23}Na quadrupole splitting for a lamellar mesophase containing egg yolk lecithin is reported in paper X. A rather complicated result is obtained, which cannot as yet be given a definite interpretation. However, recent similar investigations on the DML-cholesterol-heavy water system showed a minimum in the Na^{+} quadrupole splitting at a cholesterol concentration of approximately 15% (w/w). This result indicates that the changes of the splitting may be interpreted in terms of a release of sodium ions from the amphiphilic surfaces and an increase in the anisotropy of the bilayer as the cholesterol content is increased.

Concluding remarks

In spite of the many problems discussed in this thesis concerning the interpretation of line widths and quadrupole splittings of small ions in amphiphilic systems, it appears clear that the NMR methods considered are promising ways of studying ion interaction in amphiphilic systems and biological model membranes. Thus far, we have confined ourselves to studies of alkali and halide ions but it should be noted that it should also be possible to use these methods to investigate other ions of great biological interest such as Ca^{2+} and Mg^{2+} .

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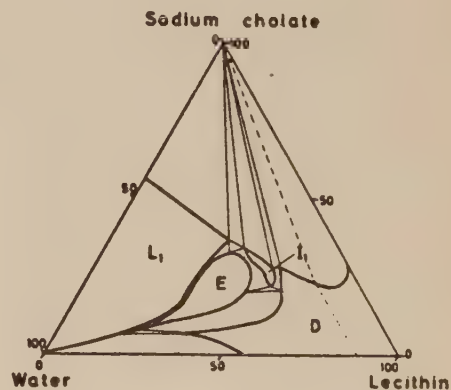
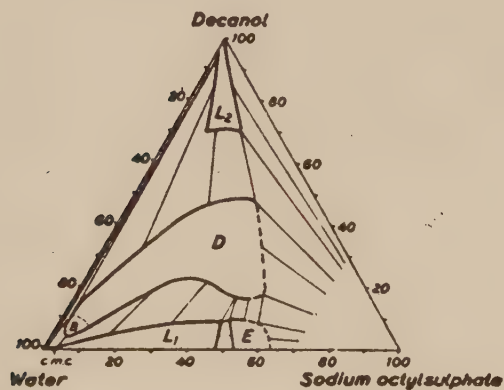
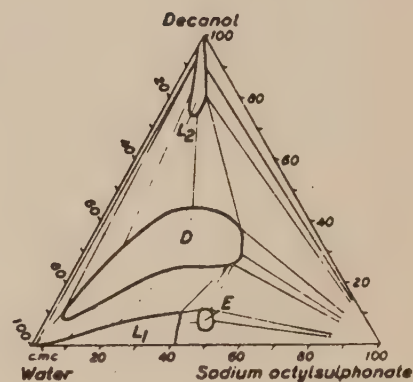
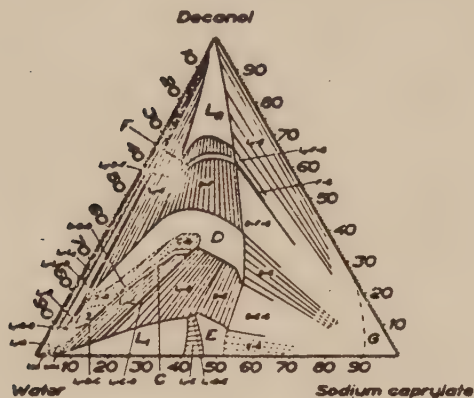
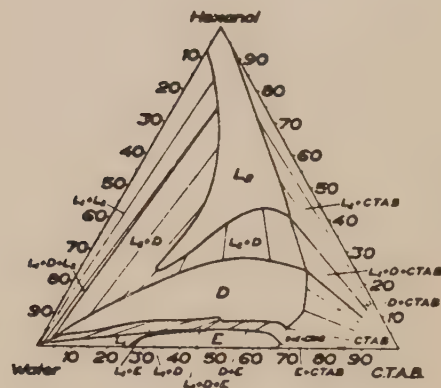
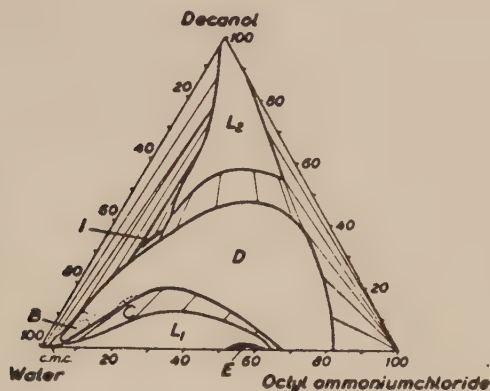
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Appendix. Phase diagrams of ternary systems (from ref. 38) used in this work.



Phase notations

L ₁ , L ₂	isotropic
B, D	lamellar
E, F	hexagonal
I	cubic
C	-

